The morphology and biology of the entomophilic *Thripinema fuscum* (Tylenchida: Allantonematidae), and the histopathological effects of parasitism on the host *Frankliniella fusca* (Thysanoptera: Thripidae)

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We used light and electron microscopy to detail the *in vivo* life cycle of the nematode *Thripinema fuscum* and to determine the effects of parasitism on tissues of the thrips host *Frankliniella fusca*. The parasitic *T. fuscum* female produced eggs within 4–5 days after ingress and the host haemocoel became packed with eggs and developing juvenile nematodes. Mature juveniles migrated to the hindgut and fully developed juveniles first emerged from the posterior end of the male or female *F. fusca* 9 days after parasitization. Parasitization induced a displacement of the host alimentary tract, an atrophy of the ovaries and fat body, and an alteration of energy and waste reserves in host tissues. Our observations reveal a specialized relationship showing that this parasite is well adapted to exploit the host digestive, reproductive and excretory systems.

**Keywords:** thrips; parasitic nematode; *in vivo* development; host–parasite interactions; histopathology

**Introduction**

Parasites have developed multiple strategies to manipulate host behaviour, morphology and physiology to enhance their survival (Thomas et al. 2010). One such parasite is the obligate nematode, *Thripinema fuscum* (Tylenchida: Allantonematidae), that specifically attacks and sterilizes its host, *Frankliniella fusca* (Thysanoptera: Thripidae). The generalized life cycle of *Thripinema* spp. has been documented (Sharga 1932; Lysaght 1936, 1937; Nickle and Wood 1964; Wilson and Cooley 1972; Reddy et al. 1982; Chizhov et al. 1995; Greene and Parrella 1995; Teulon et al. 1997; Funderburk et al. 2002). Research has shown that species of *Thripinema* induce female sterility (Sims et al. 2005). They reduce the feeding of the host thrips (Arthurs and Heinz 2003; Sims et al. 2009) and the competency of their hosts to transmit primary and secondary spread of tospoviruses (Sims et al. 2005, 2009; Sims 2010). Hence, *T. fuscum* is a key natural enemy capable of causing near extinction of local populations of *F. fusca* and reducing disease transmission in field conditions (Funderburk et al. 2002). However, the *in vivo* development of *T. fuscum* and the mechanisms that these nematodes use to manipulate the biology and behaviours of their thrips hosts are unknown.

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The goal of this research was to determine how the parasitic *Thripinema* modulates the morphology and physiology of its thrips host. An initial step to reach this goal was to examine the impact of *T. fuscum* on the *F. fusca* host using a combination of light and electron microscopy and determine the *in vivo* life cycle of *T. fuscum* and identify and describe changes to target tissues and cells affected by *Thripinema* invasion, replication and emergence.

**Material and methods**

**Laboratory colonies**

Adult *F. fusca* collected from *Arachis hypogaea* (Fabales: Fabaceae) in Citra, Florida at the University of Florida’s Plant Research and Education Unit (29°24’ N, 82°10’ W) were used to establish non-parasitized and *T. fuscum*-parasitized *F. fusca* laboratory colonies. Non-parasitized colonies were maintained as previously described (Sims et al. 2005). Thrips were parasitized by transferring 1-day-old non-parasitized *F. fusca* adult females to 1.5-ml Eppendorf microcentrifuge tubes containing a 1-cm diameter *A. hypogaea* leaf disc and two *T. fuscum*-parasitized adult *F. fusca* that were excreting nematodes. The young adults were contained with the parasitized females for 24 h, removed, and placed in individual tubes provisioned with a fresh leaf disc daily until used, as required, in the experiments. Control thrips were reared in the same manner, except they were exposed to two non-parasitized *F. fusca*.

**Light microscopy**

Non-parasitized (*n* = 30) and parasitized (*n* = 30) *F. fusca* females were immobilized on ice and dissected in a droplet of double-distilled water on a glass slide using minuten pins. Dissected specimens were photographed and measured with the AUTO-MONTAGE PRO SYSTEM (Auto-Montage Pro 5.02.0096; Syncroscopy, Frederick, MD, USA). Free-living *T. fuscum* (about 1000) were collected by rinsing 1.5-ml microcentrifuge tubes that had previously held parasitized *F. fusca* adult females with 200 µl HEPES-buffered saline (pH 6.8). The buffer from each tube was collected and pooled into a single tube, centrifuged at 5000 *g* for 3 min and exchanged with warm (∼37°C) 2.5% gluteraldehyde fixative buffered in 0.1 M sodium cacodylate containing CaCl₂ (1 mg/ml). The in vivo stages of *T. fuscum* (about 1000) were collected by dissecting parasitized *F. fusca* females (*n* = 20) in a 10-µl droplet of HEPES-buffered saline and transferring the droplet of nematodes into a 1.5-ml microcentrifuge tube containing warm fixative for 10 min. Nematodes were placed individually in a droplet of glycerin and covered with a cover slip.

**Scanning electron microscopy**

Adult female *F. fusca* (*n* = 6 non-parasitized; *n* = 8 parasitized) were immobilized on ice and adhered to double-sided tape attached to a microscope slide. A 10-µl droplet of the warm 2.5% gluteraldehyde fixative prepared as described above was immediately placed over the specimen. The submerged insect was pierced with a finely pulled capillary tube through the cuticle of each body segment to allow for the exchange of fluids. After 5 min, the specimen was transferred to a 1.5-ml microcentrifuge tube
with warm, fresh fixative for 2 h. Free-living and \textit{in vivo} stages of \textit{T. fuscum} (about 1000) were collected and fixed for 2 h at room temperature as described above. The fixed \textit{F. fusca} and \textit{T. fuscum} specimens were transferred to specimen holders that sandwich microscopic organisms between nucleopore filters (0.4-µm; Nucleopore Corp., Pleasanton, CA, USA) to reduce sample loss during fluid transitions (Kurtzman et al. 1974). Samples were washed, post-fixed, and dehydrated with a syringe (Table 1). Following the final dehydration in acetone, the syringe was removed and the specimens were dried using the critical-point method (Bal-Tek 030 CPD, Cheshire, UK). After drying, the nucleopore membranes containing \textit{T. fuscum} were attached to scanning electron microscopy (SEM) stubs with double-sided adhesive. The \textit{F. fusca} females were transferred to a copper adhesive on SEM stubs and fractured by lightly dragging

Table 1. The procedures for the preparation of \textit{Frankliniella fusca} and \textit{Thripinema fuscum} tissue for scanning electron microscopy (steps one to five) and transmission electron microscopy (steps one to six).

<table>
<thead>
<tr>
<th>Step</th>
<th>Stage</th>
<th>Chemical</th>
<th>Time</th>
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<tbody>
<tr>
<td>1</td>
<td>Fixation</td>
<td>2.5% gluteraldehyde</td>
<td>2 h at room temperature or overnight at 4°C</td>
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<td>2</td>
<td>Buffer wash</td>
<td>0.1 M cacodylate buffer</td>
<td>three times at 15 min each</td>
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<tr>
<td>3</td>
<td>Post-fixation</td>
<td>1% osmium tetroxide</td>
<td>2 h at room temperature or overnight at 4°C</td>
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<td>4</td>
<td>Water wash</td>
<td>double-distilled H₂O</td>
<td>three times at 15 min each</td>
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<td>5</td>
<td>Dehydration</td>
<td>10% ethanol</td>
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<td>6</td>
<td>Infiltration</td>
<td>25% resin/75% absolute acetone</td>
<td>4 h or overnight at room temperature</td>
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<td>50% resin/50% absolute acetone</td>
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<td>75% resin/25% absolute acetone</td>
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<td>100% resin</td>
<td>4 h or overnight at room temperature</td>
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<td>100% resin</td>
<td>6 h at room temperature</td>
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a razorblade over the surface of the insect and prying the cuticle open with fine forceps and minuten pins. The SEM stubs were coated with gold for 130 s in a sputter-coater [Denton Vacuum Desk II (Au/Pd), Moorestown, NJ, USA] and examined with a scanning electron microscope (Hitachi FE-S4000; Hitachi High Technologies America, IL, USA) operating at 10 kV.

Transmission electron microscopy
Non-parasitized (n = 124) and fully parasitized (n = 110) F. fusca females actively excreting nematodes were prepared for transmission electron microscopy (TEM) following the protocol described in Table 1. When needed, sodium bis (2-ethylhexyl) sulphosuccinate (Aerosol O.T. 100%) was used to break the surface tension and help submerge thrips in fixative. All samples were embedded in a 3–4% ultra-low-gelling agarose block (product no. A5030-1G; Sigma-Aldrich, St Louis, MO, USA) before post-fixation. Dehydrated samples were infiltrated with Epon-Araldite resin (cat no. 13940; EMS, Hatfield, PA, USA) and Z-60-40 embedding primer (cat no. 50440-10; EMS) was added to help the resin adhere to the insect cuticle. Samples were polymerized in a 65°C oven. Resin blocks were sectioned using a Reichert-Jung Ultracut E Microtome (model 701701; Depew, NY, USA). Thick sections (0.5 µm) were stained with 1% toluidine blue in 1% borax for 15 s, covered with Permount and a cover slip, and viewed under a phase contrast microscope with images collected by an RT Spot Diagnostics Imaging System (Spot Imaging Solutions, Sterling Heights, MI, USA). Thin sections (70–90 nm) were collected on formvar carbon-coated copper grids (200 mesh; EMS), post-stained in 0.5% uranyl acetate for 10 min followed by Reynold’s lead citrate for 5 min, and viewed at 75 kV with an Hitachi H-600 transmission electron microscope (Hitachi High Technologies America, IL, USA). A total of 30 non-parasitized and 32 parasitized F. fusca females were thick sectioned; of those, specific tissues were examined under the electron microscope from 10 non-parasitized and 16 parasitized F. fusca females.

Results
Life cycle and morphology of T. fuscum
There were no external detectable symptoms or morphological changes induced by development of the T. fuscum parasite to the definitive thrips host (Figure 1A). However, in vivo development of the obligate T. fuscum resulted in the conversion of internal host body mass to nematode biomass (Figure 1B–E). The complete life cycle of T. fuscum is shown in Figure 2. The infectious free-living female entered a female or male F. fusca host, and within 1–3 days, the parasitic female underwent a morphological transformation in which the slender, vermiform-shaped invasive phenotype converted to an obese, oval phenotype in the host haemocoel (Figure 2B). The female’s protective cuticle was shed and replaced by a microvilliated hypodermal layer interspersed with nodules, cuticular pits and ampullae; the stylet, oesophagus and alimentary system atrophied; and the body converted to a single swollen and convoluted ovary (Figure 3A–E, H). The maturation of the parasitic female occurred in direct apposition to the basement membrane of the host’s midgut cells (Figure 3F–H). The parasitic female produced eggs within 4–5 days after ingress and typically there were one to three eggs in her ovary at a time (Figure 3H). Eggs were oval-shaped with
Figure 1. (A) Dorsal view of a non-parasitized (right) and parasitized (left) *Frankliniella fusca* female; (B) a non-parasitized female with eggs and (C) a parasitized female with three parasitic *Thripinema fuscum* females and progeny; thick sections of (D) a non-parasitized *F. fusca* female and (E) a parasitized *F. fusca* female. Abbreviations: mg, midgut; fb, fat body; nem, nematode; ov, ovary; ves, electron-dense vesicle. Scale bars: A–C, 0.5 mm; D, E, 100 μm.

A visible micropylar process (Figure 4A, B, E), and young eggs had a thick chorion with nodules whereas mature eggs had a thin transparent chorion with a visible J1 (Figure 4B, D). Eggs were cushioned against host fat body in the haemocoele (Figure 4F), and the chorion served as a protective barrier guarding the nematode embryo against host immune factors (Figure 4G). The eggs hatched into corpulent juveniles that morphed to the characteristic vermiform juvenile shape 6–8 days after parasitization (Figure 2C–F). The young juveniles (J2 to J3) were primarily localized in the host haemocoele, although juveniles occasionally migrated to the thoracic and head tagmata. The juveniles underwent three moults in the host haemocoele and a more defined cuticle formed with each moult (Figure 5A–D). During each moult, the hypodermis separated from the cuticle and formed an exocellular space filled with collagen that was reabsorbed in the assembly of the new cuticle (Figure 5D). Host immune products were often found adhered to the cuticular surface (Figure 5B).
Figure 2. The life cycle of *Thripinema fuscum* in (A) an adult female *Frankliniella fusca* host; (B) the progressive enlargement of the parasitic female (right to left); (C) eggs (=J1) produced by the parasitic female; (D–F) J2-stage through J3-stage juveniles; (G) infectious free-living females; (H) free-living male; (G) ingress of a free-living female regenerates the cycle. Scale bar: 200 μm.

The lip region and stylet developed in third-staged juveniles (Figure 5E). Late-staged female and male juveniles migrated from the host haemocoele into the alimentary tract at the junction of the midgut and hindgut and formed a mass aggregation in a sac-like region of the hindgut lumen (Figure 6). The fully developed juveniles first emerged from the posterior end of male and female *F. fusca* 9 days after ingress of the parasitic female, hence completing the life cycle (Figure 2G, H).

The infective free-living females were straight to slightly curved when heat-relaxed with a distinct cephalic region of a stoma surrounded by four submedian lobes, an excretory system with pore located on the ventral side of the anterior region, and a well-developed annulated cuticle with transverse striations and lateral lines extending the length of the body (Figure 7A–C). The mature males were curved dorsally when heat-relaxed with a cuticle similar to the infective female also bearing lateral lines.
The stylet was indistinguishable, and the external posterior region possessed caudal alae with crenate margins, paired spicules and a thin well-sclerotized gubernaculum (Figure 7D, E).

**F. fusca–T. fuscum histopathology**

Histological examination of non-parasitized and parasitized *F. fusca* females demonstrated the internal impacts of *T. fuscum* on the thrips host. Parasitization induced displacement and invasion of the alimentary tract, atrophy of the ovaries and alteration in energy reserves, as evidenced by a reduction of fat body and glycogen and an accumulation of numerous electron-dense vesicles in the host fat body, midgut cells and Malpighian tubules (Figure 1D, E).

The SEM of fractured parasitized *F. fusca* thrips revealed numerous juvenile nematodes oriented longitudinally in the abdominal haemocoele (Figure 8A). The densely
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Figure 4. Thripinema fuscum eggs: (A) thick section of a host Frankliniella fusca female 6 days after parasitization with abdominal cavity full of nematode eggs; (B) scanning electron micrographs of T. fuscum eggs including J1 embryos visible through egg chorion; (C) transmission electron micrograph of a T. fuscum embryo curled inside chorion in the host abdomen; scanning electron micrographs of (D) protuberances on the egg’s surface and (E) aeropylar process on egg (arrow), (F) eggs cushioned within host fat body, and (G) host immune factors on the egg surface. Abbreviations: nem eggs, nematode eggs. Scale bars: A, 100 μm; B, 10 μm; C, 30 μm; D, G, 2 μm; E, 1.2 μm; F, 30 μm.

packed nematodes pressed against the host midgut, forming constrictions of the lumen and indentions on the basal side of the basement membrane (Figure 8B).

Gross dissection of parasitized thrips revealed that the ovaries of parasitized T. fuscum were half the size of those in non-parasitized thrips (Figure 1D, E; Figure 9A, D). The reduced ovarioles were significantly displaced as a result of the numerous juvenile nematodes in the haemocoel and, as a result, the zones of the oocytes were compressed against either juvenile nematodes or other host tissues (Figure 9B, E). The TEM showed a degradation of the oocytes apparent through the presence of poorly defined nuclei of different sizes in the surrounding follicular epithelium, reduction in organelle content and tears in the ladder-like extensions connecting the oocyte to the follicle cell (Figure 9C, F).

In addition to the reproductive tissues, there was a reduction in the fat deposits of parasitized F. fusca females (Figure 1D, E). The atrophied fat bodies in parasitized F. fusca lacked lipid droplets but contained numerous electron-dense spherical inclusion bodies filled with granular material (Figure 10A, C). These bodies were also located in the midgut cells of parasitized F. fusca (Figure 10B). There was a
Figure 5. Thripinema fuscum juveniles: (A) transmission electron micrograph documenting various cuticular structures; (B) scanning electron micrographs showing the host factors adhered to the cuticle surface, (C) shedding of the cuticle, (D) transmission electron micrograph of a juvenile completing ecdysis as evidenced by the shed outer cuticle (arrow) and the assembly of a new cuticle peripheral to the hypodermis, and (E) scanning electron micrograph of the developing mouthparts with visible stylet. Scale bars: A, 0.5 µm; B, E, 2 µm; C, 4.3 µm; D, 3.75 µm.

Figure 6. (A) Thick section of a Frankliniella fusca female 9 days after parasitization with an aggregation of late-staged Thripinema fuscum juveniles in the hindgut; scanning electron micrographs of a fractured F. fusca revealing (B, C) T. fuscum aggregation in hindgut with (D) a male (arrow) coiled around the females; (E) gross dissection of the F. fusca female host showing a nematode mass in the hindgut. Abbreviations: mt, Malpighian tubules; nem, nematodes. Scale bars: A, 100 µm; B, 231 µm; C, 50 µm; D, 30 µm; E, 250 µm.
Figure 7. Scanning electron micrographs of the free-living *Thripinema fuscum* female with (A) annulated cuticle with transverse striations and fully-developed mouth, (B) excretory pore on the anterior ventral surface, and (C) two lateral lines extending the length of the body. Scanning electron micrographs showing (D) the *T. fuscum* male with (E) copulatory structures including caudal alae, paired spicules and a gubernaculum. Abbreviations: ep, excretory pore; ll, lateral lines. Scale bars: A, 2.73 \( \mu \text{m} \); B, 5 \( \mu \text{m} \); C, 8.57 \( \mu \text{m} \); D, 60 \( \mu \text{m} \); E, 6 \( \mu \text{m} \).

Figure 8. Scanning electron micrographs that show (A) numerous juvenile *Thripinema fuscum* juveniles aggregated longitudinally in the female *Frankliniella fusca* haemocoele and (B) the resulting compressed host midgut (arrow) with depressions. Scale bars: A, 75 \( \mu \text{m} \); B, 30 \( \mu \text{m} \).

depletion of glycogen in many of the fat bodies as demonstrated by the space void of glycogen and organelles (Figure 10C). This depletion in glycogen coincided with an increased glycogen content inside juvenile nematodes (Figure 10D). Parasitized *F. fusca* also had areas of stored glycogen aggregated in the abdominal muscle tissue (Figure 10E–G). Lastly, the Malpighian tubules of parasitized *F. fusca* had a higher content of secretory vesicles and uric acid crystals when compared with non-parasitized *F. fusca* (Figure 10H, I).
Figure 9. A healthy *Frankliniella fusca* female with (A) a robust reproductive system with developing eggs in the ovary; transmission electron micrographs showing (B) an ovariole in a healthy female with distinct follicle cells surrounding the developing oocyte and (C) the tight ladder-like extensions between the oocyte and follicle cell, the abundance of organelles in the oocyte, and the well-defined nucleus in the follicle cell; (D) a *F. fusca* female parasitized by *Thripinema fuscum* with a reduced reproductive system with atrophied ovary; transmission electron micrographs showing (E) the displacement of host ovarioles as a result of the numerous juvenile nematodes in abdominal haemocoel and (F) the tears in the ladder-like connections between the oocyte and follicle cell, the depletion of organelles in the oocyte, and the poorly defined nucleus. Abbreviations: n, nucleus; nem, nematode; org, organelles, ov, ovariole. Scale bars: A, D, 0.5 mm; B, E, 10 µm; C, F, 2 µm.

Discussion

Our light and electron microscopy observations conducted at 24-h intervals provided detail into the life cycle of *Thripinema*. After penetrating the host through the coxal cavities or the soft intersegmental membranes (Tipping et al. 1998; Lim et al. 2001), the infective female underwent a dramatic phenotypic transformation into the parasitic female 24–72 h later. This is probably triggered by exogenous stimulation by host factors (Croll 1970; Sukhdeo and Sukhdeo 2004). Siddiqi (2000) and Sims et al. (2005) previously reported that the development of the parasitic *Thripinema* female is synchronized with the host, as demonstrated by differences in developmental time when parasitizing different stages of thrips; parasitic females entering early or late instars do not differentiate into the reproductive (ovarian) phenotype until the thrips begin to develop their adult reproductive organs. Hence, we speculate that the parasitic female uses host reproductive hormones as a trigger for maturation. Synchrony between entomophilic nematodes and their hosts has been previously noted, although the possibility that such parasites may use host endocrine factors to achieve this synchrony has not received much attention (Davey and Hominick 1973).

During the first 24–48 h after invasion, the cuticle of the *T. fuscum* female was shed and the epicuticle was transformed into a microvilliated layer with various hypodermal processes. These cuticular ampullae, nodules and pits increase the surface area of
Ultrastructural studies conducted on the integuments of various Tylenchid parasitic females have also revealed hypodermal processes with identical structure(s) and function (Riding 1970; Poinar and Hess 1972; Cliff and Baldwin 1985; Subbotin et al. 1993, 1994; Subbotin and Chizhov 1996). In all samples evaluated, the parasitic *T. fuscum* female was nestled against the host midgut where she presumably uses her absorptive hypodermal surface to sequester nutrients directly from host midgut cells. Similar to the parasitic female, juveniles may also accumulate and assimilate nutrients to aid in the absorption of nutrients required for egg production.

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for development through their cuticle using deep pits located in their exocuticle. The constriction of the midgut lumen induced by the nematodes pressing against the basal membrane of the alimentary tract may explain the previously observed feeding reductions of parasitized *F. fusca* in that the oral intake of *F. fusca* is decreased because of a reduced stomach volume (Sims et al. 2009).

In this study, we were unable to determine how *T. fuscum* evades the immune system of its host. Host immune factors were found adhered to the cuticular surface of eggs and juvenile nematodes residing in the host haemocoele, and these surface-bound antibodies were probably shed with each moult. Although not observed in the micrographs, *T. fuscum* may excrete a surface coat similar to those described in other secermentean nematodes, which is either continually shed or altered in protein composition to prevent recognition by host antibodies (Philipp et al. 1980; Blaxter et al. 1992). The late-stage juveniles had a functional stylet. Tomalak et al. (1988) reported that stylet feeding of juvenile *Neoparasitylenchus, Allantonema* and *Contortylenchus* spp. caused minor tissue damage to host beetles. In this study, nematodes absorbed nutrients directly through their cuticle and direct damage to host tissues by stylet feeding of juvenile nematodes was not observed. The function of the stylet in early J3 juveniles was to breach tissue barriers from the haemocoele into the alimentary tract in preparation for their exodus from the host (Sharga 1932; Lysaght 1936; Reddy et al. 1982). Sharga (1932) reported observing *Thripinema aptini* juveniles in the abdominal haemocoele use their stylets to bore from the midgut or oviduct to the pyriform rectum where they apparently remained for a period of time before they exited through the anus with the insect’s frass. Reddy et al. (1982) observed *Howardula (= Thripinema) aptini* exiting through the ovipositor of a *Megalurothrips* species. Juvenile *T. fuscum* were not observed in the foregut or midgut lumen of parasitized individuals, suggesting that the late-staged juvenile nematodes used their stylet to penetrate the alimentary tract through a pocket located at the pyloric valve where the proximal region of the Malpighian tubules meets the posterior midgut and anterior hindgut. Invasion of the nematodes into this junction has been documented by Serrao et al. (2008) for nematode parasites of *Hypocryphalus mangiferae*. Male *T. fuscum*, which lack a functional stylet, would also be able to enter through this thin epithelial tissue region.

After the late-staged juveniles entered the alimentary tract, they migrated to the hindgut, where the males apparently inseminate the females before their exodus from the posterior region of the host. From an evolutionary perspective, it is preferential that *T. fuscum* mate inside the host rather than outside in the harsh environment where they are prone to isolation and desiccation. Mating within the host insect has been reported for *Parasitylenchus*, an Allantonematid Tylenchid parasite of *Drosophila recens* (Perlman and Jaenike 2001). Often, more than one parasitic *T. fuscum* female parasitized a thrips host, which we believe reduces consanguinity in the nematode populations. Additionally, *Thripinema* has a 19 : 1 (female : male) sex ratio, suggesting a tendency towards parthenogenesis in the absence of males (Sims et al. 2005).

Our light and electron microscopy provided insight into the interactions occurring *in vivo* between the parasite and its *F. fusca* host. Parasitism by *T. fuscum* caused substantial pathological changes to female *F. fusca* host tissues and organs. The most pronounced change induced by *Thripinema* in their thrips host is a reduction in the size and shape of the reproductive organs (Lysaght 1937; Greene and Parrella 1995; Loomans et al. 1997; Arthurs and Heinz 2003). *Thripinema fuscum* causes a rapid sterilization of parasitized thrips (Sims et al. 2005). The time frame to induce sterility
depends on the stage that is parasitized; adult female *F. fusca* parasitized as larvae do not produce any eggs whereas females parasitized as adults stop laying eggs within 2–3 days (Sims et al. 2005). The physiological mechanisms driving sterility in this genus are not well understood. Sterility may result from *Thripinema* deprivating thrips of the protein(s) required for normal development or secreting a toxin that damages the reproductive organs, from *Thripinema* biomass signalling stretch receptors to halt oogenesis, diversion of host resources, or from *Thripinema* symbionts (= Wolbachia) interacting with host reproductive tissue (Lysaght 1937; Greene and Parrella 1995; Sims 2010; Sims et al., in preparation). Sims et al. (2005) concluded that the parasitic *T. fuscum* female is responsible for stopping oogenesis because sterility is induced before the production of juveniles in the host haemocoele.

Numerous additional families within the Nematoda have been reported to induce partial or complete sterility in their insect host [e.g. Sphaerulariidae and *Musca autumnalis* (Treece and Miller 1968), *Scolytus ventralis* (Ashraf and Berryman 1970a,b), *Vespa simillima* (Sayama et al. 2007); Allantonematidae and *Scolytus* spp. (Oldham 1930), *Dendroctonus pseudotsugae* (Thong and Webster 1975), *Hypothenemus hampei* (Castillo et al. 2002); Neotylenchidae and *Sirex noctilio* (Bedding 1972)], but mechanisms for the observed sterility have not been determined. Perlman and Jaenike (2003) cross-infected *Drosophila* spp. with different allopatric and sympatric *Howardula* spp. and concluded that sterility is host determined because an individual host response to infection (measured by degree of sterility) was the same regardless of the infecting nematode species. Roseler and Roseler (1973), who determined that the yolk proteins of parasitized queen *Bombus terrestris* are of a lower concentration than those of the non-parasitized conspecifics, suggested that the nematode causes injury to the yolk-producing corpora allata gland. *Tenebrio molitor* beetles parasitized by the rat tapeworm exhibit reduced fertility and fecundity and an increased lifespan (Hurd 2001). The metacestodes produce a small effector peptide(s) that stimulates the transcription of vitellogenin messenger RNA but suppresses translation resulting in decreased levels of vitellogenin in parasitized *Tenebrio* females (Webb and Hurd 1999; Warr et al. 2006). In addition, components transiently present in the haemolymph retard the uptake of yolk proteins by the ovarian follicles (Major et al. 1997). Studies have suggested that the unidentified effector(s) disrupts hormonal activity (Webb and Hurd 1995). In addition to inhibiting vitellogenin synthesis, the tapeworms also induce re-absorption of the developing eggs as result of the induction of an apoptotic programme in the fat body cells (Warr et al. 2005, 2006). Similarly, parasitized *F. fusca* females had a significant reduction in fat body and there was evidence of apoptosis in the follicle cells surrounding the ovarioles. Based on this observation, *T. fuscum* nematodes may deplete the fat body, which reduces the synthesis of vitellogenin necessary for oocyte development and maintenance.

In addition to alterations to the host reproductive structures, there were changes to the energy and waste reserves in parasitized *F. fusca* females. Fat bodies were reduced and replaced with large electron-dense vacuoles resembling storage proteins. Storage proteins are produced in the fat body and released into the haemocoel where they are reabsorbed primarily by fat bodies and stored as protein granules until they are used (Wang and Haunerland 1991). These vesicles were distributed throughout the thrips and were localized primarily under the dorsal cuticle where fat body accumulates, and in midgut cells. The storage vesicles diminished with increased nematode biomass (e.g. as parasitism progressed) and it is unknown whether these storage proteins are
nematode-derived or host-derived and utilized. There was also an obvious depletion of host glycogen in the fat bodies that was simultaneous with an observed glycogen increase in juvenile nematodes. The transfer of glycogen from *F. fusca* to *T. fuscum* suggests that the parasite uses host glycogen as a carbohydrate/glucose source for energy. Parasite-induced depletion of host fat body tissue is a common outcome of parasitism (see Bailey and Gordon 1973; Condon and Gordon 1977; Schmidt and Platzer 1980; Tomalak et al. 1984). Cheng and Snyder (1962) reported that cells recently involved in glycogen digestion have aggregates of glycogen granules that form amorphous masses in the cytoplasm of host snail cells. As trematode parasitism progresses, host cell glycogen is depleted and an increase of glycogen within the parasites is observed. Decreased levels of glycogen or glycogenesis in fat body have also been reported for *Schistocerca gregaria* infected with *Mermis nigrescens* (Gordon and Webster 1971), larval black flies *Prosimulium miuxtum, Prosimulium fuscum* and *Simulium venustum* infected with *Neomesosermis flumenalis* (Condon and Gordon 1977), mosquito *Culex pipiens* infected with *Romanomermis culicivorax* (Schmidt and Platzer 1980), and *Lymantria dispar* infected with the microsporidium *Vairimorpha* sp. (Hoch et al. 2002). Finally, *T. fuscum* excreta in the haemolymph of parasitized *F. fusca* females was filtered through the Malpighian tubules in the form of storage vesicles and uric acid crystals.

Hence, the morphological changes to the *F. fusca* host as a result of parasitism by *T. fuscum* are great. Parasitism induces a displacement of the host alimentary tract, an atrophy of the ovaries and fat body, and alterations to the energy and waste reserves. Congruent with these changes in its host, the infectious female of *T. fuscum* undergoes a dramatic morphological transformation to the parasitic form. In short, the female parasite becomes a reproductive machine that is adapted to use host digestive, reproductive and excretory systems for its survival and reproduction. The changes in internal morphology suggest that host physiology related to the reproductive, digestive, circulatory and excretory systems is likely to be affected as a result of parasitism. Changes in the morphology and physiology of the digestive and circulatory systems could directly affect the ability of the host to acquire and transmit plant diseases and so inhibit their primary spread, while changes to the host reproductive system might affect the secondary spread of such diseases.

Results from this research provide a step towards understanding how insect parasitic nematodes interact with their hosts *in vivo*. This provides a basis for developing a unique biological control system to better regulate this important vector of plant viruses. Potentially, elucidation of the mechanisms responsible for inducing sterility in parasitized thrips may provide novel avenues for regulating the intrinsic rate of increase of this pest insect. Likewise, identifying damage to host tissues resulting from parasitism that reduces Tospovirus competency (acquisition and transmission) may also provide targets that suppress disease spread.

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